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Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy

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Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. We studied the effects of fish oil on the progression of renal insufficiency in rats with subtotal nephrectomy. Five weeks after a 1-2/3 nephrectomy, sixteen rats were fed two different diets which differed only in fat composition. Lipid in the control diet was primarily beef tallow; that of the experimental diet, menhaden oil. Fish oil–fed rats had significant increases in plasma creatinines, decreases in urinary PGE_2 and accelerated death rates. An additional twelve rats underwent 1-1/3 nephrectomies, and the same dietary manipulations, followed by renal clearance, histologic and biochemical studies after 12 weeks on the diets. Fish oil–fed rats again did worse, with decreased glomerular filtration rates and filtration fractions, more proteinuria and more glomerular sclerosis. Glomeruli and slices of cortex, medulla and papillae from rats fed fish oil produced much less PGE_2 and TXB_2 than dietary controls. Fish oil–induced suppression of renal PGE_2 may be deleterious in this model and may outweigh the beneficial effect derived from TXA_2 suppression. In contrast to fish oil's potentially therapeutic role in cardiovascular and immune–mediated renal disease, this diet is detrimental in rat renoprival nephropathy. This illustrates the importance of examining the effects of fatty acid manipulation individually for each disease entity.

Prostaglandins, specifically PGE_2 and PGI_2, help maintain renal blood flow and glomerular filtration in clinical conditions associated with renal compromise, and thus are thought to be beneficial [1–3]. Conversely, thromboxane A_2 (TXA_2), another cyclooxygenase metabolite, may contribute to the evolution of certain renal diseases [2, 3].

Eicosanoids must be derived from polyunsaturated fatty acids ingested in the diet. These fatty acids cannot be synthesized de novo by mammals [4]. Arachidonic acid (AA), the conventional precursor, contains four double bonds with its terminal double bond at the "omega minus 6" position. Eicosapentaenoic acid (EPA), found in fish, contains five double bonds with its terminal double bond at the "omega minus 3" position [4]. AA is readily converted to the "2" or diene series of cyclooxygenase metabolites; EPA may be converted to the "3" or triene series [5–8]. TXA_2 is a potent vasoconstrictive [4], platelet aggregatory and leukocyte chemoadhesive agent [9], EPA-derived TXA_3 is biologically inert [5]. PGI_3 however, has been reported to be equipotent to PGI_2 [5]. It has also been shown that EPA is converted to leukotriene B_5 (LTB_5) [10, 11] which is much less biologically active than AA-derived LTB_4 [10].

Greenland Eskimos, whose diets are rich in EPA, have abnormal platelet function, prolonged bleeding times, and less cardiovascular disease [12–14]. These changes correlate well with the reduction in biologically active TXA_2 [5]. Conclusions gained from these population and in vitro analyses have served as the rationale for the use of fish oil in the prevention of cardiovascular disease [14, 15], with less attention to other organ systems, specifically the kidney. Several studies have shown that a fish oil–enriched diet prevents proteinuria in two different models of genetic systemic lupus erythematosus [16, 17]. Another study, performed in humans, demonstrates that fish oil supplements preserve renal function in IgA nephropathy [18].

The objectives of our study were a) to determine the effects of a fish oil–enriched diet on the course of the nonimmune–mediated renal disease produced by surgical renal ablation and b) to assess the effect of this dietary manipulation on renal PGE_2 and TXB_2 accumulation. We thought that the fish oil–enriched diets might halt the progression of this renal disease, but contrary to our expectations, in two separate experiments fish oil was detrimental, resulting in the acceleration of both functional and histological renal deterioration. We conclude that such a dietary manipulation should not be used indiscriminantly, but rather should be directed to specific diseases.

Methods

Surgery

Subtotal nephrectomies were performed on adult, female Sprague–Dawley rats (Camm, Wayne, New Jersey, USA). Through an abdominal incision, the right kidney was removed. The left kidney was then exposed. For animals in Group I (16 rats) both the upper and lower poles were ligated tightly, resulting in a 1-2/3 nephrectomy. For the twelve rats in Group II, the right kidney was removed and only one pole of the left kidney was ligated, resulting in a 1-1/3 nephrectomy. In order to simulate a true clinical situation in which therapy is not initiated until the disease is established, a five week interval was allowed

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Table 1. Fatty acid analysis of dietary lipids

Fatty acid	Menhaden oil EPA-enriched diet	Beef tallow Control diet	Safflower oil To provide essential fatty acids
14:0	8.2	2.7	0.11
16:0	13.5	23.9	6.38
16:1	13.5	5.3	0.57
18:0	5.5	17.7	2.45
18:1	12.5	41.2	11.9
18:2	5.2	6.1	73.3
20:3 (DHG)	1.4	<0.05	—
20:4 (AA)	1.5	<0.05	—
20:5 (EPA)	14.4	<0.05	<0.05
18:3, 22:5, 22:6	14.0	—	—

Numbers represent percent of the total fatty acid content.

between the renal ablative procedure and initiation of the experimental diets.

The first experiment (Group I) was a pilot study, the goal of which was to see if fish oil–enriched diets had any effect on the course of renoprival nephropathy. Because significant differences in plasma creatinines between fish oil–fed and control diet–fed rats were noted after 10 to 12 weeks (**Results**), in the second set of experiments (Group II), formal renal clearance measurements, renal segmental eicosanoid production and histologic analysis were performed at this time point. In order to perform these studies before the Group II rats were clinically uremic, the less extensive ablative procedure was performed.

Diets (Table 1)

During the five-week interval between surgery and initiation of experimental diets, rats were fed standard chow (Ralston Purina, St. Louis, Missouri, USA). They were then divided into two dietary subgroups with similar plasma creatinines and pair–fed diets which differed only in the composition of fat. Throughout the dietary study rats were housed individually in metabolic cages.

The basic diet consisted of a fat–free power (TD 84010) prepared by Teklad Test Diets, Madison Wisconsin, USA. To this was added the dietary fat, either in the form of melted beef tallow (ICN Nutritional Biochemicals, Cleveland, Ohio, USA) for the rats fed the control diet or, for the rats fed the fish oil–enriched diet, whole menhaden oil, a rich source of EPA and other omega-3 polyunsaturated fatty acids (Table 1). This menhaden oil was highly refined and processed with more than 99.5% of its fatty acids in triglyceride form. All nutrients, minerals, and vitamins were equal in each diet and in compliance with recommended nutritional requirements [19]. The composition of both regular and experimental diets after the addition of fats was: carbohydrate, 50% wt/wt; protein, 19% wt/wt; fat, 21% wt/wt; cellulose 5%, wt/wt; minerals (Teklad, AIN-76, 170915), 4% 2/2; and vitamins (Teklad, 40060), including Vitamin E to prevent oxidation of the fatty acids while in the chow, 1% wt/wt. Sufficient safflower oil was added to the diets to provide equal amounts of linoleate and arachidonate. All oils were stored in a refrigerated room under nitrogen.

Biologic measurements

Every two weeks tail pressures were recorded while animals were under light ether anesthesia, and blood was collected for plasma creatinine, hematocrit, and platelet count. Twenty–four hour urine collections were made on these days for PGE_2, TXB_2, creatinine, and protein determinations, at which time animals were only allowed access to water. Plasma and urine creatinine were determined by the Jaffe reaction, measuring both true creatinine and non-creatinine chromagens. Radioimmunoassays for urinary PGE_2 and TXB_2 were performed on unextracted urine samples stored at −20°C [20]. Urinary protein measurements were determined by sulfosalicylic acid.

Clearance studies

DTPA and hippuran studies were performed on rats in Group II (those with the 1-1/3 nephrectomy) after 12 weeks on the diets (17 weeks postoperatively). Under ether anesthesia, flexible catheters were inserted into the femoral artery, vein, and bladder. An intravenous infusion of 2 ml/hr normal saline (20 U sodium heparin/ml) was maintained. After the rat awoke a stabilization period of approximately one hour was allowed. The following isotopes were then injected into the venous catheter: Technetium ^{99m}Diethylenetriamine pentaacetic acid, 17 μCi, (^{99m}Tc DTPA, MediPhysics, Emeryville, California, USA) to measure glomerular filtration rate (GFR) [21] and Iodohippurate Sodium, 10 μCi, (Hippuran ^{131}I Mallinkrodt, Inc., St. Louis, Missouri, USA) to measure renal plasma flow (RPF) [22]. After injection of the tracers, blood samples were collected in heparinized capillary tubes at 5, 10, 15, 20, 30, 40, 60, and 80 minutes. Radioactivity versus time was plotted semilogarithmically and clearances calculated. This single injection method has been shown to yield results which correlate well with simultaneously–performed standard clearances of cold inulin and PAH [23]. After completion of the clearance study animals were sacrificed, blood obtained for lipid and chemical determinations, and the remnant kidney removed for analysis.

Preparation and incubation of renal tissue for PGE_2 and TXB_2 measurement

All preparation of renal tissue was carried out in ice–cold normal saline. Slices of 0.5 mm thickness were made using a Stadie–Riggs microtome. Several slices from each kidney were fixed for light microscopic analysis. The remaining slices were either further separated into cortical, medullary, or papillary segments, or used for preparing glomeruli. Glomeruli were isolated by a sequential sieving technique, as reported [24, 25]. This technique does not isolate severely sclerosed glomeruli, as they tend to be fibrotic, imbedded in the cortex and do not freely pass through the sieve. The resulting preparation consisted of >95% glomeruli, the majority unencapsulated. The average time between sacrifice of the rat and division of the kidney into all these segments was no more than 90 minutes.

For incubation studies, the following buffer was used: NaCl 135 mEq/liter, KCl 10 mEq/liter, sodium acetate 10 mEq/liter, Tris pH 7.4 20 mEq/liter, glucose 5 mm/liter. Fatty acid–free bovine serum albumin (1 mg/ml) (Sigma) and Ca^{++} (2 mM) were also included. Individual renal slices or glomerular aliquots (approximately 500 to 800 glomeruli/aliquot) were resuspended

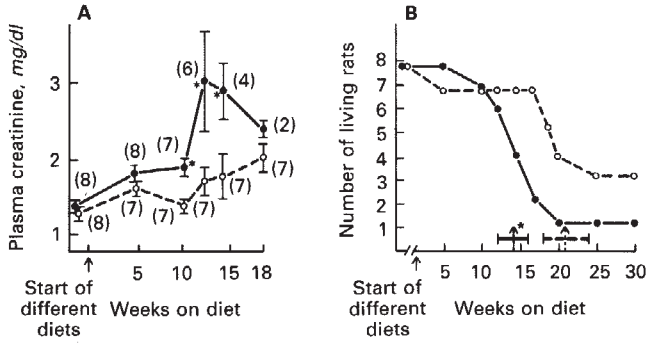

Fig. 1. *Effects of fish oil–enriched vs. control diets on renal function and survival of the group I (1-2/3-nephrectomized) rat.* **A.** Points depict mean ± SEM plasma creatinine over an eighteen week period. Parentheses indicate number of alive rats. **B.** Points depict survival of these rats. Arrows perpendicular to the ascissa denote mean ± SEM survival. Symbols are: (●) Fish oil-enriched diet; (○) control diets. (* = $P < 0.05$ EPA-enriched diet vs. control diet.)

in one ml of buffer. Immediately prior to the experiment, the incubation medium was replaced with fresh medium. Experiments were then performed in a water bath at 37° for 10 minutes with buffer alone, or buffer plus 2 μM of the divalent cation ionophore A23187 (Sigma). At the end of the incubation, supernate was aspirated and saved at −20°C for later eicosanoid measurement. Renal tissue was washed and resuspended in 1 N NaOH for protein determinations [26].

Radioimmunoassay (RIA) for PGE_2 and TXB_2

RIAs were performed on unextracted supernates and urines. Samples were diluted from 1:50 to 1:100 and assayed at two or more dilutions. PGE_2 antibody was from the Institut Pasteur, Paris; TXB_2 antibody was purchased from Seragen. Cross–reactivities of these antibodies with other diene eicosanoids are published elsewhere [25]. In our laboratory cross–reactivity of the PGE_2 antibody with PGE_3 is 8%. Radioactive ligands (^{3}H)PGE_2 (specific activity 120 to 170 Ci/mmol and (^{3}H)TXB_2 (specific activity 180 Ci/mmol) were purchased from Amersham Radiochemicals, Arlington Hts., Illinois, USA. Buffer without renal tissue was incubated in parallel experiments and served as a blank. This blank never exceeded 5% of the experimental values.

Preparation of tissue for light microscopic analysis

Renal slices from each rat were fixed in 10% neutral formalin, dehydrated in alcohol and imbedded in paraffin. Four μm sections were then stained with Hematoxylin–Eosin (H-E). Masson–Trichrome stain was used in the evaluation of fibrosis.

Measurement of glomerular diameter

H-E stained sections were observed with an oculomicrometer. Only those glomeruli that presented an apparent cross section were examined. Two perpendicular diameters of each glomerulus were measured in nanometers and their average was considered as the individual glomerular diameter. All sections of each kidney were evaluated for glomerular sclerosis. The extent of tubular dilatation and atrophy, interstitial fibrosis, vascular thickening, and nephrocalcinosis, was expressed as "absent" (0), "questionable" (1), "mild" (2), "moderate" (3), or "severe" (4). Two pathologists evaluated the tissue separately. At least 50 glomeruli per rat were evaluated histologically. All these examinations were done in a blinded manner.

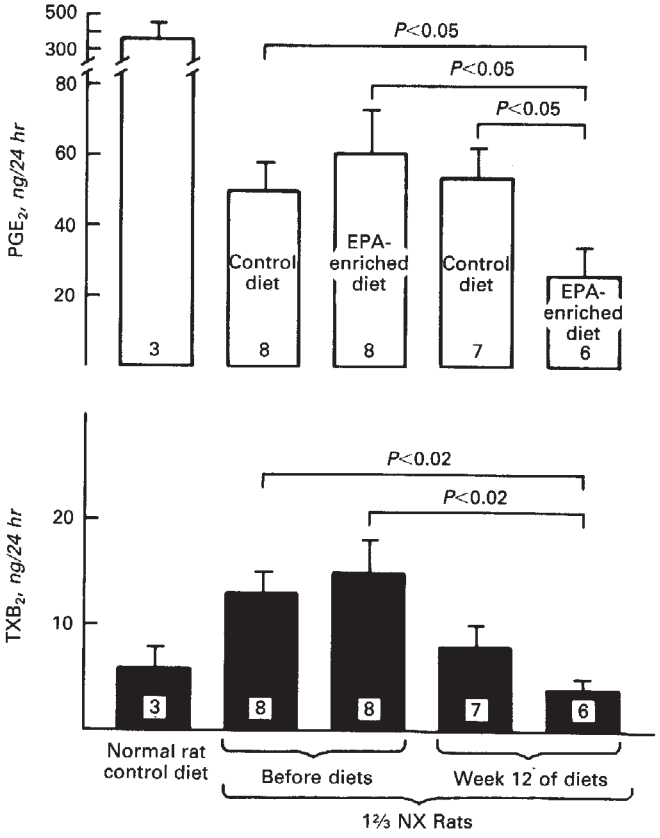

Fig. 2. *Effect of control vs. fish oil–enriched diets on radioimmunoassayable urinary TXB_2 and PGE_2 in group I (1-2/3NX) rats.* Bars depict mean ± SEM. Numbers within the bars represent number of rats.

Measurement of plasma lipids

Cholesterol was measured by the Fermco enzymatic method for free cholesterol and cholesterol esters (Fermco Biochemical, Elk Grove Village, Illinois, USA). Triglycerides were measured enzymatically using the Worthington Diagnostic Reagent Set (Cooper Biomedical, Diagnostic Division, Malvern, Pennsylvania, USA).

Statistics

All results are reported as mean ± SEM. Statistical analysis used was the Student's *t*-test. Whether the analysis was paired or unpaired is indicated in **Results.**

Results

Group I (rats with 1 2/3 NX)

General parameters. After 12 weeks on the experimental diets (17 weeks post-NX) there were no differences in the blood pressures (mean ± SEM) (control diet 129 ± 6 mm Hg vs. fish oil–enriched diet 133 ± 18 mm Hg), weights (control diet 291 ± 17 g vs. fish oil–enriched diet 286 ± 5 g) or proteinuria (control diet 78 ± 13 mg/24 hr vs. fish oil–enriched diet 79 ± 20 mg/24

Table 2. Effects of fish oil–enriched vs. control diet on the 1-1/3 nephrectomized rat (Group II)

	Weeks on diet					
	0 5 wk p NX	2 7 wk p NX	4 9 wk p NX	7 12 wk p NX	10 15 wk p NX	12 17 wk p NX
BP *mm Hg*						
Control diet	149 ± 10	162 ± 20	142 ± 16	113 ± 5	100 ± 8	102 ± 14
Fish oil diet	134 ± 8	153 ± 10	138 ± 7	118 ± 6	115 ± 4	125 ± 12
	NS	NS	NS	NS	NS	NS
Weight *g*						
Control diet	236 ± 9	270 ± 6	284 ± 5	289 ± 4	286 ± 3	293 ± 8
Fish oil diet	226 ± 9	269 ± 5	278 ± 7	280 ± 7	280 ± 7	294 ± 8
	NS	NS	NS	NS	NS	NS
Hematocrit %						
Control diet	45 ± 1	40 ± 2	42 ± 3	47 ± 1	46 ± 1	47 ± 1
Fish oil diet	45 ± 2	43 ± 1	43 ± 2	46 ± 1	45 ± 1	47 ± 2
	NS	NS	NS	NS	NS	NS
Platelets × *1000*						
Control diet	814 ± 184	998 ± 202	642 ± 197	588 ± 164	619 ± 165	588 ± 132
Fish oil diet	1,201 ± 282	1,240 ± 469	602 ± 158	586 ± 145	549 ± 134	530 ± 127
	NS	NS	NS	NS	NS	NS

Numbers represent mean ± SEM; $N = 6$; NS = not significant

hr). Fish oil–fed rats had a gradual drop in hematocrit during the dietary study which was significantly different from dietary controls ($P < 0.02$) at week 14 of the diets (control diet 42 ± 1% vs. fish oil–enriched 34 ± 3%). Because post-mortem examinations revealed no evidence of infection or blood loss in the fish–oil fed rats, we attributed the anemia to their elevated creatinines. We did not perform statistical comparisons of blood pressure, proteinuria, hematocrit or weight beyond fourteen weeks after initiating the experimental diets because so few rats eating the fish oil–enriched diet were alive.

Renal function. Figure 1A depicts the progression of plasma creatinine concentration versus time. Before initiation of diets, mean plasma creatinine of the rats to be fed fish oil–enriched diets as well as those to be fed the control diet (± SEM) was 1.3 ± 0.1 mg%. Rats fed fish oil diets subsequently suffered a more rapid rise in plasma creatinine. This became statistically significant by week 10 to 12 on the diet (15 to 17 weeks postoperatively).

Survival. After initiation of the experimental diets, the rats fed control diets lived an additional 21 ± 3 weeks (mean ± SEM, Fig. 1B). Those fed the fish oil–enriched diet survived a significantly shorter time ($P < 0.05$), 14 ± 2 weeks (mean ± SEM). During the two weeks prior to death, all rats ate less and lost weight.

Urinary PGE_2 and TXB_2. Before initiation of the diets, there was no significant difference in 24 hour urinary PGE_2 (rats to be fed fish oil 61 ± 15 ng vs. those to be fed control diet, 50 ± 8 ng). These values are markedly reduced from normal, non-nephrectomized rats analyzed at the same time (360 ± 90 ng, $N = 3$). However, when urinary PGE_2 is normalized to creatinine clearance, renal PGE_2 production by nephrectomized rats exceeded that of normal rats (150 ± 14 μg PGE_2/cc/min vs. 83 ± 27 μg PGE_2/cc/min). After 12 weeks on the diet, the urinary PGE_2 of the rats on the control chow had not changed, but the urinary PGE_2 of the rats fed fish oil was markedly reduced (Fig. 2). This reduction was significant whether compared to the rats on the control diets at week 12 or to EPA-fed rats prior to the initiation of diets.

Table 3. Radionuclide clearances (week 12), and protein excretion (before diet and at week 12 of diet) of 1-1/3 nephrectomized rats

	Control diet $N = 5$	Fish oil diet $N = 6$	P
^{131}I hippuran clearance			
Renal Plasma flow ml/min/rat	2.91 ± 0.55	3.87 ± 0.50	NS
Tc^{99m} DTPA clearance			
glomerular filtration rate *ml/min rat*	1.09 ± 0.15	0.72 ± 0.12	<0.05
Filtration fraction *GFR/RPF*	0.37 ± 0.08	0.19 ± 0.02	<0.05
Proteinuria *mg/24 hr*			
beginning of diet	18 ± 6	20 ± 8	NS
Week 12 of diet	38 ± 10	81 ± 22	<0.05

Numbers represent mean ± SEM; NS = not significant

Urinary TXB_2 five weeks after subtotal nephrectomy was similar in the rats to be fed control or fish oil–enriched diets (13 ± 2 ng and 15 ± 2 ng, respectively) which is significantly greater than non-nephrectomized rats (6 ± 2 ng). Rats fed the fish oil–enriched diet subsequently had a significant reduction in urinary TXB_2 over the study period.

In summary, we found the following in the pilot study: (1) plasma creatinines rose more quickly in 1-2/3 NX rats fed fish oil–enriched diets than in those rats on the control diet; (2) fish oil fed rats were more anemic. We attribute this to their more severe renal insufficiency; (3) urinary PGE_2 and TXB_2 were significantly reduced in fish oil–fed rats; and (4) fish oil–fed rats did not live as long as their dietary controls.

Group II

General parameters. After 12 weeks on the diet (Table 2), rats with less extensive renal ablation (1-1/3 NX) all had similar weights (fish oil–fed rats, 294 ± 8 g; control diet rats, 293 ± 8 g), hematocrits (fish oil–fed rats, 47 ± 2% control diet rats 47 ± 1%), and platelet counts (fish oil–fed rats, 530,000 ± 126,700; control diet rats 588,000 ± 132,000). The hematocrits of all Group II rats were higher than those in Group I. We attribute

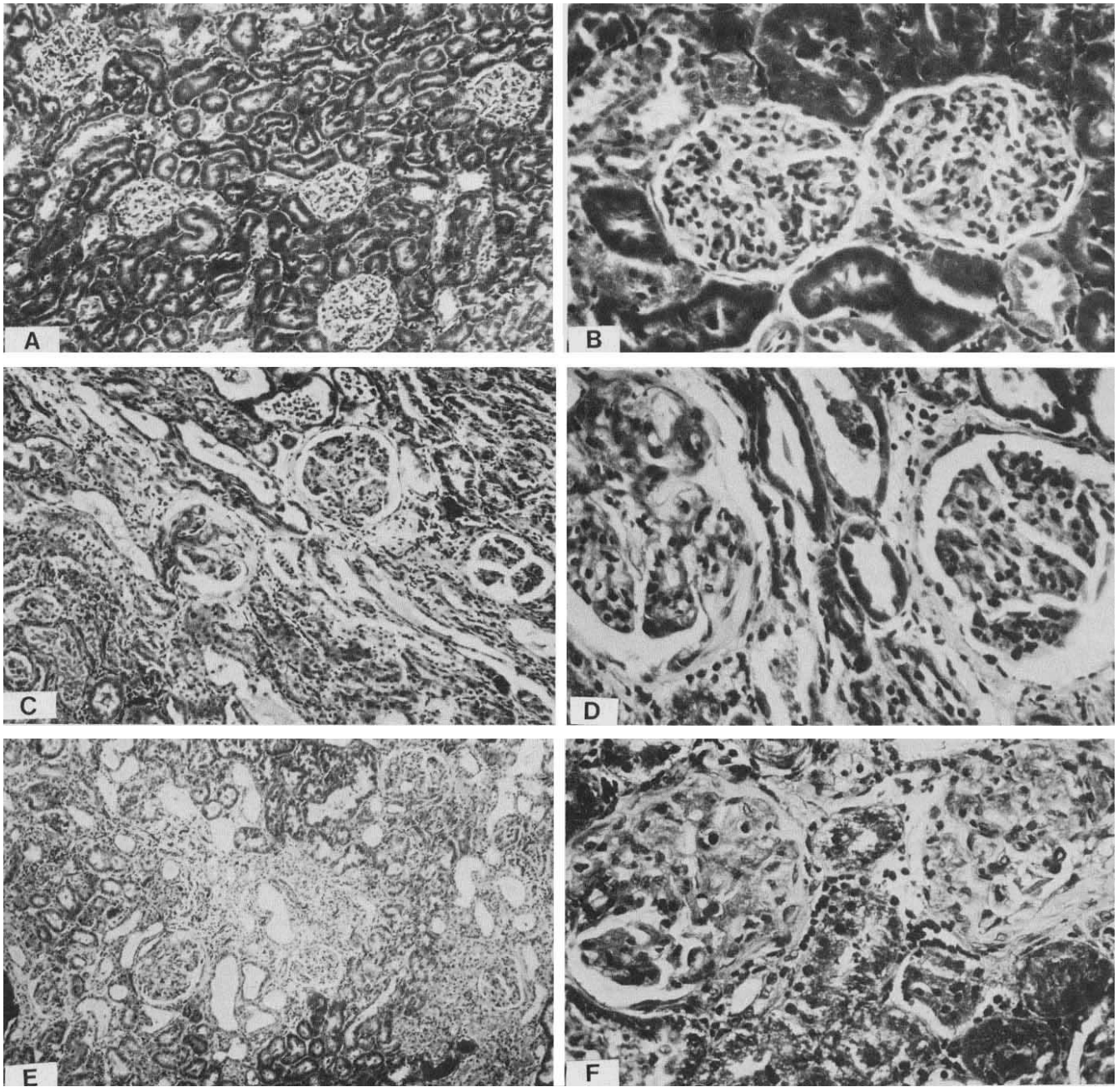

Fig. 3. *Histopathology of remnant renal segments from fish oil–fed rats vs. dietary controls (Group II).* Masson Trichrome Stain. **A** and **B**: Renal tissue from normal age–matched unperfused kidney (A ×100, B ×250). All glomeruli appear normal and the interstitium is devoid of infiltrating cells. **C** and **D** are from 1-1/3 nephrectomized rats fed normal diets. C shows interstitial fibrosis with tubular dilatation and atrophy (C, ×100). D (×250) shows two glomeruli, one with glomerular adhesions and focal sclerosis. **E** and **F** are from 1-1/3 nephrectomized rats fed fish oil–enriched diets. The interstitial disease is slightly more severe (E, ×100), however, the major changes occur in the glomeruli, more of which are totally sclerosed (F, ×250).

this to a less extensive renal ablation and hence less severe renal functional impairment than Group I. Blood pressures in Group II, although slightly elevated, were lower than in Group I. By the twelfth week on the diets, tail pressures were slightly higher in the fish oil–fed rats than in their dietary controls (fish oil–fed rats, 125 ± 12 mm Hg; control diet rats, 102 ± 14 mm Hg).

Renal function. At the time rats were paired for initiation of experimental diets, mean plasma creatinine (± SEM) was 1.1 ± .1 mg% (Table 3). This value is less than that of the Group I rats. At the time of the clearance studies the mean plasma creatinines of the rats in Group II, regardless of diet, were not significantly different. Proteinuria at the beginning of the diets was the same. Twelve weeks after the initiation of the diets, the rats fed fish oil had significantly more proteinuria than their dietary controls (81 ± 22 mg/24 hr vs. 38 ± 10 mg/24 hr).

Table 4. Light microscopic analysis of remnant kidneys from 1-1/3 NX rats fed fish oil–enriched vs. control diets (Group II)

	Normal rat	Control diet 1-1/3 NX $N = 5$	Fish oil diet 1-1/3 NX $N = 6$
Glomerulus			
Diameter *um*	103 ± 1	138 ± 2	137 ± 2
Percentage of totally sclerosed glomeruli	0	1.8% ± 1.1%	9.9% ± 2.0%[a]
Tubules			
Dilatation (0–4)	0	2.2 ± 0.5	2.5 ± 0.2
Atrophy (0–4)	0	1.4 ± 0.5	1.6 ± 0.4
Interstitial inflammation in cortex, medulla (0–4) (Lymphocytes, Macrophages)	0	2.4 ± 0.6	2.6 ± 0.5
Fibrosis (0–4)	0	1.8 ± 0.3	2.0 ± 0.5
Vessel thickening (0–4)	0	1.0 ± 0	1.3 ± 0.2
Nephrocalcinosis (0–4) and Tubular Destruction	0	2.4 ± 0.5	2.0 ± 0.5

A similar analysis of a kidney from an age–matched, normal rat on the control diet is included. Approximately 50 to 60 glomeruli per rat were examined. 0 = absent; 1 = questionable; 2 = mild; 3 = moderate; 4 = severe. Numbers represent mean ± SEM for each group. N = number of kidneys examined.

[a] $P < 0.05$ fish oil–enriched vs. control diet kidney

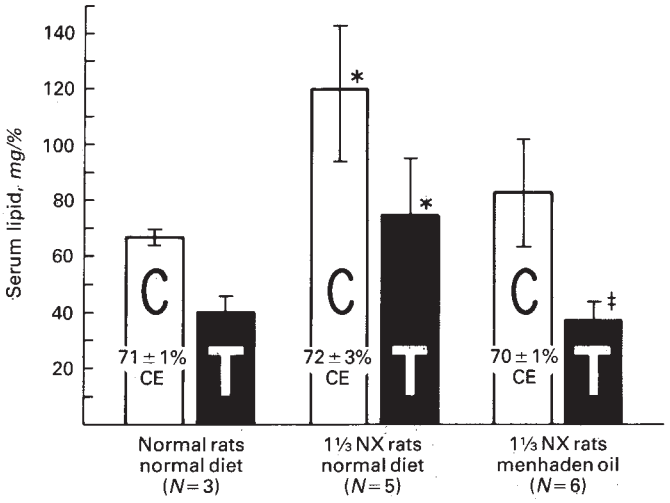

Fig. 4. *Plasma lipids.* Bar graphs show mean ± SEM for cholesterol ("C," clear bar) and triglycerides ("T," solid bar). Normal rats on standard chow diets (bars on left) are shown for comparison. Numbers in clear bar represent percentage of cholesterol esters (CE). * = $P <$ 0.05, compared to normal rats; $P < 0.05$, compared to nephrectomized rats on normal diets.

One control rat died an anesthetic related death just prior to the clearance study. Statistics were performed on the remaining eleven rats using Student's unpaired t-test, shown in Table 3. (^{131}I) hippuran plasma flow was slightly higher in the rats fed fish oil (3.87 ± 0.50 cc/min vs. 2.91 ± 0.55 cc/min). However, (TC^{99m}) DTPA clearance of the fish oil–fed rats was significantly lower than control (0.72 + 0.12 cc/min vs. 1.09 + 0.15 cc/min). These values also held true when expressed as "cc/min/g kidney" or "cc/min/100g rat weight." Filtration fraction, calculated as (TC^{99m}) DTPA clearance/(^{131}I) hippuran plasma flow, was therefore markedly decreased in the fish oil–fed rats (0.19 ± 0.02) versus the dietary controls (0.37 ± 0.08).

Renal pathology. Shown in Figure 3 are representative micrographs of renal remnants of a rat on the control (3C and D) and fish oil diet (3E and F). Micrographs of a kidney from a normal non-nephrectomized, age–matched control examined at the same time are also shown (3A and B).

Results of the quantitative, blind analysis of all renal tissue are shown in Table 4. The glomerular diameters of all rats in Group II, independent of dietary manipulation, were greater than diameters from normal rats, but there were no differences between the rats on the two diets (fish oil, 137 ± 2 μm; dietary control, 138 ± 2 μm, normal non-nephrectomized, 103 μM). Of the 50 to 60 glomeruli per rat examined almost all glomeruli regardless of diet had some degree of distortion or focal sclerosis; however, global sclerosis was much more prominent in the fish oil–fed rats than in dietary controls (9.9% ± 2.0% vs. 1.8% ± 1.1%). Chronic inflammation, interstitial fibrosis, and vascular thickening were slightly increased in the fish oil–fed rats. The degree of tubular dilatation, atrophy and nephrocalcinosis was not significantly different.

Plasma lipids. It has been suggested that hyperlipidemia may contribute to the progression of glomerular sclerosis [27–29]. The 1-1/3 NX rats on control diets had significant increases ($P < 0.05$) in both plasma cholesterol (120 ± 26 mg% vs. 67 ± 3 mg%) and triglyceride (75 ± 20 mg% vs. 40 ± 6 mg%) compared to non-nephrectomized rats analyzed at the same time (Fig. 4). In contrast, both cholesterol and triglyceride levels remained normal in 1-1/3 NX rats fed the fish oil–enriched diet. Fish oil–induced reduction in triglycerides has been reported in humans with hyperlipidemia [30].

Renal eicosanoid production. Table 5 depicts basal and ionophore–stimulated radioimmunoassayable PGE_2 and TBX_2 production by isolated glomeruli and slices from cortices, medullae and papillae of the remnant kidneys. Production of these eicosanoids by segments from normal, age–matched non-nephrectomized rats in our laboratory is shown in the left column.

PGE_2 production by glomeruli, cortices, and medullae, but not papillae, of the partially nephrectomized rats on control diets significantly exceeded that of normal rats. Enhanced PGE_2 synthesis by remnant glomeruli has been reported by others [31, 32]. The lack of enhanced papillary PGE_2 production may relate to the lower interstitial osmolality in the diseased kidney and, therefore, less stimulation of PGE_2 synthesis [33]. Incubation in 2 μM A23187 resulted in two– to fourfold stimulation of PGE_2 by the kidneys of normal rats, but did not stimulate the renal remnants from partially nephrectomized rats on the control diet. This lack of ionophore stimulation suggests that there is maximal stimulation of PGE_2 synthesis even under basal conditions in the remnant kidney.

The fish oil diet significantly reduced basal PGE_2 production by all segments of the remnant kidneys compared to corresponding renal segments from the partially nephrectomized rats on the control diet (Table 5). Papillary PGE_2 production by rats fed the fish oil–enriched diet was also significantly less than normal rats. Similar to renal segments from the partially ne-

Table 5.

	Normal Rat Control (n = 4)		1-1/3 NX Rat Control Diet (n = 5)		1-1/3 NX Rat Fish Oil Diet (n = 6)	
PGE_2 *ng/mg/protein/10 min*						
Glomeruli						
Basal	5.2 ± 0.7	< 0.01	26 ± 8[a]		6 ± 2[c]	
+A23187 2 *uM*	10.0 ± 0.5		30 ± 8		10 ± 3[c]	
Cortex						
Basal	1.6 ± 0.3	< 0.01	28 ± 8[a]		8 ± 3[c]	
+A23187 *2 uM*	8.3 ± 2.2		30 ± 10		7 ± 1[c]	
Medulla						
Basal	13 ± 4	<0.05	115 ± 31[a]		36 ± 8[c]	
+A23187 *2 uM*	34 ± 8		121 ± 32		38 ± 7[c]	
Papilla						
Basal	2,270 ± 811	< 0.05	780 ± 214		284 ± 95[b,c]	
+A23187 *2 uM*	8,199 ± 2,013		705 ± 253		266 ± 120[b,c]	
TXB_2 *ng/mg protein/10 min*						
Glomeruli						
Basal	0.8 ± 0.3	< 0.05	0.8 ± 0.1	< 0.05	0.2 ± 0.1[b,c]	< 0.05
+A23187	1.9 ± 0.3		1.8 ± 0.4		0.5 ± 0.1[b,c]	
Cortex						
Basal	Undetectable	<0.01	1.1 ± 0.3		0.4 ± 0.1[b,c]	
+A23187	0.3 ± 0.1		1.3 ± 0.4[a]		1.0 ± 0.5[b]	
Medulla						
Basal	0.1 ± 0.1	< 0.01	4 ± 1[a]		0.7 ± 0.3[b,c]	
+A23187	0.5 ± 0.3		4 ± 1[a]		1.2 ± 0.5[b,c]	
Papilla						
Basal	4 ± 1		8 ± 2		2 ± 1[b,c]	
+A23187	10 ± 4		9 ± 2		3 ± 1[b,c]	

[a] $P < 0.05$ 1-1/3 NX rat, control diet vs. normal rat, control diet
[b] $P < 0.05$ 1-1/3 NX rat, fish oil diet vs. normal rat, control diet
[c] $P < 0.05$ 1-1/3 NX rat, fish oil diet vs. 1-1/3 NX rat, control diet
Eicosanoid production by segments of renal remnants from 1-1/3 NX rats (Group II) on control vs. fish oil–enriched diets. Renal segments from normal kidneys of age–matched rats on control diets are depicted in the left column.

phrectomized rats on the control diet, however, the remnant renal segments from the fish oil–fed rats did not respond to ionophore.

In contrast to the enhanced glomerular PGE_2 production by partially nephrectomized rats on the control diet 17 weeks after surgery, glomerular TXB_2 production at this time was not different from normal. The ionophore caused a doubling of TXB_2 production by both preparations (Table 5). Cortical and medullary TXB_2 synthesis by the remnant kidney of rats fed the control diets remained enhanced and failed to respond to the ionophore. This enhanced cortical and medullary thromboxane production by the remnant kidney may reflect the presence of activated macrophages in the interstitium of this model (Table 4).

The fish oil–enriched diet significantly reduced basal TXB_2 production by remnant glomeruli, cortices and medullae compared to corresponding renal segments from partially nephrectomized rats on control diets. However, cortical and medullary TXB_2 production by fish oil–fed nephrectomized rats was still greater than that by a normal non-nephrectomized rat and failed to respond to the ionophore.

In summary, we found the following in the 1-1/3 nephrectomized rat: Twelve weeks after the initiation of experimental diets, rats fed the fish oil–enriched diet had significantly worse functional and histologic renal deterioration than their dietary controls. This happened in spite of a reduction in cholesterol, triglycerides, and glomerular thromboxane production. Cortical and medullary TXB_2 production was less than that of the dietary controls, but still enhanced compared to a normal rat. PGE_2 production by remnant glomeruli, cortices and medullae from rats on the control diets was significantly elevated compared to non-nephrectomized controls even seventeen weeks after surgery. The fish oil diet significantly reduced PGE_2 in these tissues to levels no different from a normal rat.

Discussion

Renal ablation in the rat results in glomerular sclerosis and death [27, 34]. The factors responsible for progressive renal failure in this model are incompletely understood [35], but it has been suggested that hypertension, both systemic and intraglomerular [36], intraglomerular microthrombus formation [37], with thromboxane release [31], and hyperlipidemia [27–29] may all contribute to glomerular destruction. Of note, the method we used to achieve renal ablation, that of ligation or excision of part of the kidney [38–40] is not associated with the degree of hypertension seen when the renal vessels are ligated [36]. Fish oil may exert an antiplatelet effect [5, 13–15], reduce serum lipids [30], suppress the production of biologically active thromboxane [5, 41, 42, 44], and, perhaps, lower blood pressure [42]. We reasoned, therefore, that dietary enrichment with fish oil would be beneficial in this model. We found the opposite. In two separate experiments a fish oil–enriched diet was detrimental.

The effects of "omega-3" fatty acids, which are abundant in fish oil, have been attributed in part to alterations in eicosanoid metabolism. There is uniform agreement that they cause a reduction in the amount of diene cyclooxygenase products [41–46]. Conversion of omega-3 fatty acids to triene cyclooxygenase products has also been studied [5, 7–9, 45] and they are not converted as readily as arachidonate [5, 8, 45]. It has even been questioned whether the amount of triene cyclooxygenase metabolites formed is enough to be of physiologic significance [8]. It has been more recently reported that EPA can also be converted by the lipoxygenase pathway to LTB_5, which has markedly less chemotactic ability than LTB_4 [10, 11].

Prickett, Robinson and Steinberg [16] and Kelley et al [17] found that the NZB/NZW, F_1 and MRL-1pr mouse, fed from birth the same fish oil–enriched diet as ours, survived longer than their dietary controls and did not develop proteinuria. In a prospective study, Hamazaki, Tateno and Shishido reported that "EPA" capsules, administered to men with IgA nephropathy and moderate renal insufficiency, stabilized serum creatinines compared to dietary controls [18]. In contrast, we find the opposite to be true in surgical renal ablation in rats.

Why would a fish oil diet be beneficial in some diseases, yet detrimental in another? It may, in part, be due to its effects on eicosanoid synthesis. Both systemic lupus erythematosus and IgA nephropathy are immune–mediated and inflammatory in origin. Surgical renal ablation is not. The factors initiating glomerular damage in these diseases are different. Immune–mediated diseases are associated with activation of the lipoxygenase pathway within the glomerulus [2, 3]. There is no good evidence of lipoxygenase activation in non-immune disease. Fish oil may be beneficial in immune–mediated disease because of simultaneous effects on the lipoxygenase pathways (to produce a biologically attenuated LTB_5), and cyclooxygenase pathways (to inhibit TXA_2 synthesis). The progression of immune- and nonimmune–mediated renal disease to glomerulosclerosis may share common factors [47], but if the immune–mediated renal disease remains active, the elements which initiated the renal damage, including the lipoxygenase pathway, continue to be present.

Both immune–mediated and nonimmune–mediated renal disease, including renal ablation, activate the cyclooxygenase pathway within the glomerulus and stimulate glomerular or renal TXB_2 synthesis [1, 2, 31, 32, 48]. The source of thromboxane synthesis may be the glomerulus itself, but other potential sources are activated platelets and/or inflammatory cells. Chronic administration of a thromboxane synthase inhibitor by Purkerson et al for five weeks following subtotal renal ablation resulted in functional and histologic improvement [31]. In contrast to the beneficial effects found by Purkerson, a similar study by Stahl, et al, using the same model, had no beneficial effect [32]. Others, however, have found thromboxane suppression to be beneficial in both immune–mediated [2, 3, 48], and nonimmune–mediated renal disease [49, 50].

Fish oil diet did reduce the amount of TXB_2 assayed in every segment of the renal remnant, but fish oil supplementation and pharmacologic thromboxane synthase inhibition cannot be regarded as equivalent therapies. Our diet not only reduced thromboxane, it also markedly inhibited the production of PGE_2 by each segment of the renal remnant. These findings are in agreement with others who find that EPA inhibits production of AA-derived cyclooxygenase metabolites [41–46]. Adequate production of prostaglandins, specifically PGE_2 and PGI_2, helps to maintain glomerular filtration in clinical conditions associated with renal compromise, and thus are thought to be beneficial [1, 2]. Our present study supports this belief. This suggests that a major effect of fish oil supplementation, suppression of renal PGE_2 production, is an untoward side effect and outweighs any beneficial effect derived from thromboxane inhibition.

We purposely did not initiate the fish oil diets until five weeks after surgery in order to simulate a true clinical situation in which therapy is not initiated until disease is present. Whether pretreatment or perioperative initiation of the fish oil diet would be beneficial or detrimental cannot be deduced from the results of this study. We did not want to study the feasibility of fish oil as a preventive therapy, but rather as an active intervention.

Fish oil supplementation is not the only fatty acid manipulation which has been tried in different renal diseases with conflicting results. Hurd et al reported that a diet enriched with linoleic acid, the major arachidonate precursor, had no effect on the clinical course of renal disease in the NZB/NZW, F_1 mouse [51]. Barcelli, Weiss and Pollak [40] and Heifetz, Purkerson and Klahr [52] found that this same diet preserved renal function in the partially nephrectomized rat. In Barcelli's study, the high linoleate diet resulted in elevated PGE_2, but not TXB_2, production by remnant cortices, and elevated TXB_2, but not PGE_2 production by remnant medullae. Regarding dietary fatty acid manipulation in renal disease, it is interesting to note that, lupus, the renal disease which responds favorably to fish oil, does not respond to linoleate supplementation [51], and that the progressive insufficiency induced by renal ablation, which is attenuated by linoleate supplementation [40, 52], is actually accelerated by fish oil supplementation.

Dietary fatty acid manipulation is potentially both a feasible and powerful therapeutic maneuver [53]. To assume that all the effects of dietary fatty acid manipulation are eicosanoid–mediated, however, is simplistic. For example, the mechanisms by which fish oil reduces serum lipids [30], alters the endocytic response of macrophages [54], suppresses tumor growth [46], and alters mitochondrial membrane electron–transport [55], are not understood. One must remember that dietary polyunsaturated fatty–acids are directly incorporated into cell membranes and may affect any cellular event mediated or regulated by cell membrane phospholipids. Ours is the first study which reports that one type of fatty acid manipulation, that with fish oil, may be detrimental in a specific disease model, subtotal renal ablation in the rat. It emphasizes the importance of performing studies which elucidate the exact mechanism(s) by which dietary fatty–acid manipulation exerts its effects. In conclusion, dietary fatty–acid manipulation should not be recommended as a global therapy but, instead, may have to be tailored for specific diseases.

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Appendix. Abbreviations

AA	Arachidonic acid
DTPA	Diethylenetriamine pentaacetic acid
EPA	Eicosapentaenoic acid
GFR	Glomerular filtration rate
H-E	Hematoxylin–eosin
Kf	Ultrafiltration coefficient
LTB_{4-5}	Leukotriene B_{4-5}
NX	Nephrectomy (nephrectomized)
PGE_{2-3}	Prostaglandin E_{2-3}
RIA	Radioimmunoassay
RPF	Renal plasma flow
TXA_{2-3}	Thromboxane A_{2-3}
TXB_{2-3}	Thromboxane B_{2-3}

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